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Polymorphism in exon 13 of estrogen hormone receptor (*Erα*) gene by PCR-RFLP in Murrah/ graded Murrah buffaloes

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Abstract
The present study was undertaken with 204 buffaloes during June, 2016 to July, 2017 from different locations *viz*., Saraswathi Krishi Vigyan Kendra, Karur district, Tamil Nadu; Post Graduate Research Institute in Animal Sciences (TANUVAS), Katupakkam, Tamil Nadu; Central Cattle Breeding Farm, Alamadhi, Chennai, Tamil Nadu; Buffalo Research Station, Venkataramanna Gudem, S.V.V.U, West Godavari District, Andhra Pradesh and Farmers herd in Namakkal, Tamil Nadu to determine the genetic variation. The animals were genotyped by Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP). In the PCR reaction the primers used were 5' AGC ATC TAT ACA GCA TGA AGT G 3' and 5' GTG TAC TAA GCT GAC AAA GTC TTG 3'. An 870 bp long *ER*-α (exon 13) gene PCR product was genotyped for polymorphic pattern using *Mbo*I restriction enzymes. The PCR-RFLP of *ER*-α gene (exon 13) showed 614 and 256 bp long digested PCR products in all the tested samples. Monomorphic pattern as *AA* genotype for all the tested animals indicates the fixation of this allele in Murrah buffaloes.

Keywords: Murrah/Graded Murrah Buffaloes, *ERα* gene (exon 13), PCR-RFLP, Monomorphic condition

1. Introduction
In an agrarian country like India, Livestock plays an indispensible role in the food security of the country providing milk, meat and eggs. Most of the rural population is still depended on livestock sector for their livelihood. We are well aware that India is one among the 12-mega biodiversity hot spots in the world and harbour excellent livestock diversity in the form of species, breeds and strains [13].

Among 194 million buffaloes present all around the globe nearly 58% is donated by our country which comes around 108.7 million as per 19th census [10]. There are 13 well-defined and registered breeds of buffaloes in India as per the report of National Bureau of Animal Genetic Resources (NBAGR), Karnal. As per records, world's total milk production is around 800 million tonnes during 2015 within which almost 100 million tonnes is the contribution from buffalo milk [10]. Two-third of the total global buffalo milk is produced by our country. More than 50% of our nation's milk production is from buffalo population. However, low reproductive efficiency is dramatically affecting the buffalo productivity [10]. Inactive ovaries, late maturity, seasonal cycling, silent heat, prolonged postpartum interval, anoestrus and poor expression of heat signs are the major reproductive deficiencies shown by Indian buffalo. This may be due to poor management, nutrition or diseases [6, 14].

Optimal reproduction and disease control are main factors for the profitability from buffalo husbandry. Management practice, disease and genetic constituent of the animal influence more on reproductive function [4]. Even though advance techniques are practiced to control reproductive diseases in cattle and buffaloes, infertility remains a major economic problem to the farmers and its incidence appears to be rising in India also [7]. However the impact of infertility in terms of economic losses in Indian livestock is not available.

Hormones and their respective receptors play important roles in the reproductive performances in field buffaloes. Estrogen has great link with reproductive traits and it has been proved that it can affect growth, differentiation and function of reproductive organs [1, 15]. Estrogen binds with its receptors coming under nuclear receptors super family and there by regulates the gene expression [13]. Due to its role in reproduction, estrogen receptor gene is considered as

candidate marker for fertility traits in farm animals. Two forms of estrogen receptor *i.e.*, α and β are present in mammals [2, 12].

Genetic improvement of buffalo for its production traits depends on concepts of quantitative genetics of economic traits to promote more efficient and relatively easy selection of Indian buffaloes [3]. Candidate gene approach can predict more accurate breeding values and it accelerates the genetic gain achieved by selection [9]. In the present study, the polymorphism of exon 13 of estrogen hormone receptor (ERα) gene by PCR-RFLP was analyzed as a genetic marker candidate for reproductive traits in Murrah / graded Murrah buffaloes.

2. Materials And Methods

The research study was carried out during June 2016 to July 2017 with 204 buffalo blood samples were collected from organised farms *viz*., Saraswathi Krishi Vigyan Kendra, Karur district, Tamil Nadu; Post Graduate Research Institute in Animal Sciences (TANUVAS), Katupakkam, Tamil Nadu; Central Cattle Breeding Farm, Alamadhi, Chennai, Tamil Nadu; Buffalo Research Station, Venkataramanna Gudem, S.V.V.U, West Godavari District, Andhra Pradesh and Farmers herd in Namakkal, Tamil Nadu. Collected samples were brought in ice to the laboratory and stored at -20°C till processed. Genomic DNA was extracted from whole blood by using the standard high salt method [5]. UV-V is spectrophotometers used to quantify and assess purity of DNA and diluted to the working concentration of 100ng/µl, which is suitable for polymerase chain reaction (PCR). A region of estrogen receptor α (*ER*α) gene of exon 13 was amplified by using a set of forward (5′ AGC ATC TAT ACA GCA TGA AGT G 3') and reverse (5′ GTG TAC TAA GCT GAC AAA GTC TTG 3') primers. The reaction of PCR was carried out in 25µl volume containing 12.5µl of 2 X PCR master mix, 9.5µl of nuclease free water 1µl of each primer (10 pM) and 1µl of genomic DNA. PCR conditions were as follows: denaturation at 94°C for 5 min followed by 35 cycles shared in denaturation at 94°C for 45 sec, annealing at 60°C for 50 sec, extension at 72°C for 50 sec and final extension 72°C for 7 min (Bio-Rad T^{100}) [13]. The PCR products were checked by agarose gel electrophoresis to confirm the amplification before analysing for polymorphism. The PCR amplified product of exon 13 of *ER*α was digested with *Mbo*I restriction enzymes to analyse the polymorphic pattern. The restriction mixture consists of 10 units of restriction enzyme, 10X restriction buffer and Nuclease free water and its mixed with 8µl of PCR product and incubated overnight at 37°C. About 10µl of the digested samples were separated on two per cent agarose gel in 1 X TAE buffer containing ethidium bromide at 2 V/cm for one hour to determine the genotypes. The gels were visualised and the images were documented in a gel documentation system (Bio-Rad Gel Doc™).

3. Results and Discussion

The 870 bp amplified PCR product of the *ERα* (exon 13) are genotyped for polymorphic pattern using *Mbo*I restriction enzyme revealed 614 and 256 bp fragments representing *AA* genotype in all individual animals (Fig. 1). The fixation of *A* allele and absence of allele *B* in buffaloes was evident.

All the samples under study showed bands of 614 and 256 bp corresponding to the *AA* genotype (Fig. 1). In Egyptain buffaloes, two genotypes namely *AG* and *GG* with frequency of 0.18 and 0.82 were reported [8]. Due to absence of genetic variation in this studied population as similar to our study, Central Institute for Research on Buffaloes, Hisar, the similar monomorphic pattern of *ER*α (exon 13) was obtained with *Mbo*I restriction enzyme in Murrah buffalo [13].

In Murrah buffaloes, the genetic variation of 870 bp *ER*α (exon 13) gene by *Stu*I and *Hpa*II restriction enzymes were analysed [10] and two fragments at 305 bp and 565 bp by *Stu*I and 759 bp and 111 bp by *Hpa*II. Both of these enzyme digestions revealed monomorphic condition in all animals and its indicating there was no variation in the population as like our study. In PCR-RFLP analysis of 870 bp *ER*α (exon 13) gene by *Mbo*I restriction enzymes showed one migration pattern and characterizing the monomorphic condition in bovine and swine [11, 15, 16, 17] as like that of Murrah / Graded Murrah buffaloes.

The Murrah and graded Murrah buffaloes included in the present study was monomorphic for *ERα / Mbo*I and hence association studies with reproductive traits could not be carried out.

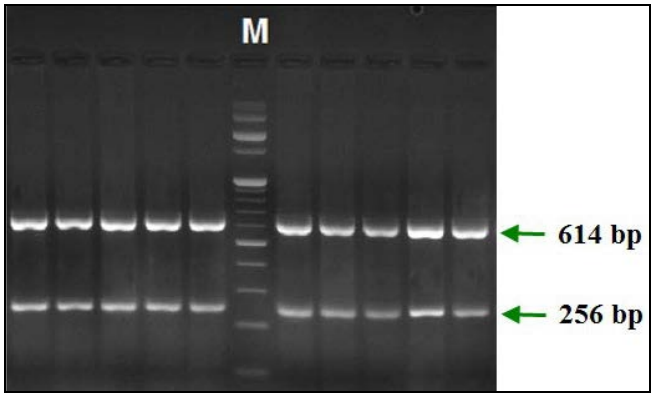

Fig 1: RFLP patterns of *ERα* (exon 13) / *Mbo*I gene in Murrah / Graded Murrah buffaloes (M - 100 bp DNA Marker).

4. Conclusion

The gene that codes for Alpha receptor of Estrogen, more specifically exon 13 showed a monomorphic pattern in all the 204 tested buffaloes with fragments of 614 bp and 216 bp length on digestion of the PCR product of size 870 bp using with restriction enzyme *MboI*. Based on the already available literature this is genotyped as *AA*. Genotype AA indicates that the *ER*α gene is fixed in the buffalo population. As this is monomorphic, *ER*α (exon 13) can be considered as a unique feature that may have relation with the reproductive characteristics of buffaloes in the studied population. To generalize these findings the study needs to be extended to other buffalo population as well.

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